REPRODUCTIVE AND MOLTING CYCLES IN CAVE CRAYFISH

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In the epigean environment, crayfish of temperate North America are subjected to annual fluctuations of many factors including organic material, various dissolved chemicals in the water, temperature and length of photoperiod. The latter two exhibit striking changes, rising in the spring from winter minima to summer maxima and lowering in the fall to the winter minima. The effects of changing temperature and photoperiod on reproductive cycles have been documented for many vertebrate and invertebrate animals.

For the majority of North American crayfishes that have been studied the pattern of annual reproduction is for breeding to occur predominantly during the fall, while egg-laying occurs during the spring after temperatures begin to rise and photoperiods are increasing. This pattern has been reported for *Orconectes obscurus* and *Cambarus diogenes* (Ortmann, 1906), *O. limosus* (Ortmann, 1906; Crocker, 1917), *O. propinquus* [= *Cambarus propinquus*] (Van Deventer, 1937; Bovbjerg, 1952; Crocker, 1957) and *C. longulus* (Smart, 1962). An annual cycle of spermatogenesis in *C. montanus* (Word and Hobbs, 1958) and of ovarian development in *C. longulus* (Smart, 1962) has been demonstrated. Molting of adult crayfish usually occurs twice a year, in late spring (spring molting period) and early fall (summer or fall molting period). Such a pattern of molting has been reported for *O. immunis* [= *Cambarus immunis*] (Tack, 1941), *O. limosus* and *O. obscurus* (Ortmann, 1906), *O. propinquus* [= *Cambarus propinquus*] (Van Deventer, 1937; Crocker, 1957), *C. longulus* (Smart, 1962) and *C. montanus* (Word and Hobbs, 1958).

Experiments by various investigators (Word and Hobbs, 1958; Lowe, 1961; Stephens, 1952; Stephens, 1955) have shown that light and temperature do affect reproductive and molting cycles of crayfish. Therefore, it is of considerable interest to examine reproductive and molting activities in cave crayfish living in a subterranean environment where there is no light and where yearly temperature changes may be small.

Materials and Methods

The data presented in this paper were collected during the years 1960–65 from the population of cave crayfish, *Orconectes pellucidus inermis*, living in Shiloh Cave near Bedford, Indiana; studies were conducted in the cave during several months of each year. These crayfish frequent the pools and slow-moving areas of the meandering stream that flows through the cave. The size of the population was measured by the Petersen proportional method (Dice, 1952). It is small in Shiloh Cave compared to the size of crayfish populations observed in epigean streams, and was estimated to be about 200 large juveniles and adults during the fall of 1962.

The number of crayfish sampled on any one date varied from 7 to 100 for the 26 trips to the cave during this study. The amount of stream habitat available for study is over 1100 meters in length with an average of 1.4 meters in width. Depth normally varies from 0.1 to 1.0 meter but may increase considerably after flooding periods. The water is clear during normal flow but increases in turbidity during flooding periods, due to silt suspension and influx of organic material. Annual records of water temperature varied by only 1° C.; the low of 12.2° C. was recorded in December and the high of 13.2° C. in August.

The reproductive cycle of females was studied by observing when females carry eggs, when newly hatched crayfish are present in the stream, and when changes in the ovary and cement glands occur (cement glands secrete material for gluing the eggs to the abdominal appendages, the pleopods). The ovary can be observed through the translucent carapace. Changes in the testes cannot be observed through the carapace; thus, the reproductive cycle of males was studied from histological sections. Testes were fixed in alcohol-formalin-acetic acid (AFA) mixture during several months of the year and, after the usual histological procedures, stained by either Mallory's triple connective tissue procedure (Guyer, 1953) or Delafield's hematoxylin and eosin. In addition the change in ratio of breeding to non-breeding adult males (differences in these males are discussed below), which is an important means for evaluation of periodic molting and reproductive activities, was compiled on each trip to the cave.

The molt cycle was categorized according to the classification of Carlisle and Knowles (1959) and Carlisle (1960): premolt, ecdysis, postmolt and intermolt. Several criteria were used to stage crayfish in the molt cycle including (1) occurrence of ecdysis, (2) relative hardness of the exoskeleton and (3) relative darkness of the exoskeleton. Animals shedding the exoskeleton are rarely seen because ecdysis occurs within an hour; however, the individual has a very soft exoskeleton for about a day before and after ecdysis. It is flexible when touched for a few weeks before ecdysis and for a week or more after ecdysis. This flexibility occurs before molting when calcium is being removed from the old shell and after molting before calcium deposition in the new shell is completed. Body pigments are lacking in *O. p. inermis;* the exoskeleton is white after ecdysis, but darkens with time due to the deposition of materials suspended in the water and perhaps to changes within the exoskeleton itself. In addition to these criteria crayfish were marked with fingernail polish during the fall months as an aid in clarifying a fall molting period.

Female Reproductive Cycle

Information about the reproductive cycle of cave crayfish is sparsely recorded in the literature. Park, Roberts and Harris (1941) reported the occurrence of one ovigerous female (carrying eggs on the abdominal appendages) on June 26 and two during the middle of September. According to them a breeding periodicity in *O. p. pellucidus* appears to be poorly developed. Giovannoli (1933) reported that ovigerous females are found during the winter; from this observation Brown (1961) assumed that an annual reproductive rhythm was present in this cave crayfish. However, Giovannoli's information was taken from Hay (1903, p. 232): "Eggs are said to be laid during the winter, but the guides were rather indefinite

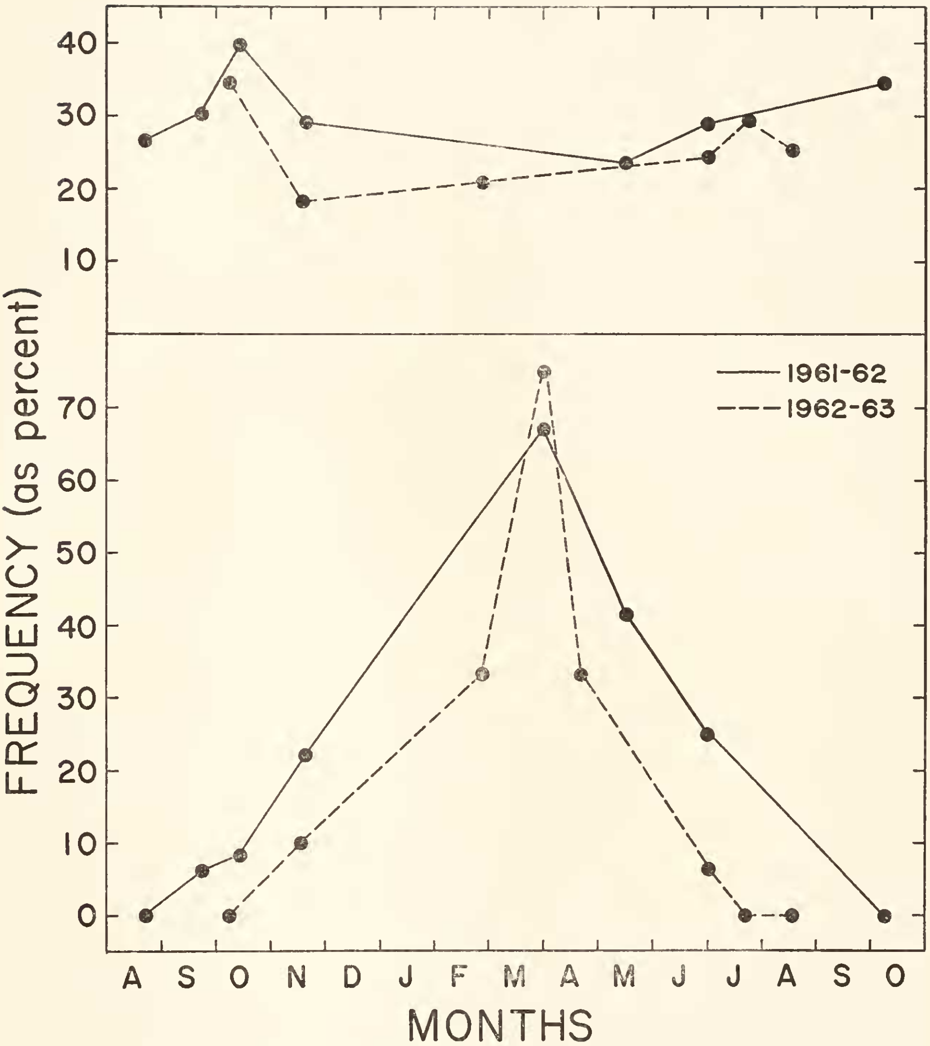

FIGURE 1. The frequency of adult females having large, yolky oocytes in the ovary, stage 4, (bottom) and the frequency of immature crayfish <18 mm. carapace length (top) in the population of *O. p. inermis* in Shiloh Cave. The number of adult females and of immature animals varied, respectively, from 3 to 24 and from 12 to 37 in samples taken during the two years.

as to the exact time." Poulson (1964) suggested that *O. pellucidus* may have a fall breeding period.

OBSERVATIONS ON FEMALE REPRODUCTIVE CYCLE

Five ovigerous females were found in Shiloh Cave, all from June 30 to August 21. An ovigerous female collected on August 16, 1963, had the most advanced embryos, and these embryos required less time to develop in the laboratory at 15–17° C. than embryos from the other females. Upon hatching the young crayfish remain attached to the adult until after the second molt; they are less than 5 mm. in carapace length after leaving the adult and entering the stream population. A few small crayfish (<12 mm. carapace length) were found during all of the months except January, March and April, but they are most numerous and conspicuous during the summer and fall months; the largest number was recorded during August, 1964.

A good indicator of an annual cycle of reproduction in a crayfish is the cycle of oocyte formation and growth in the ovary. The ovary in crayfish is a trefoil organ situated between the floor of the pericardial sinus and alimentary canal. In *O. p. inermis,* the quiescent ovary lies in a plane nearly parallel to the dorsal and ventral surfaces of the carapace. But the anterior lobes grow upwards as the ovary increases in volume, nearly reaching the dorsal carapace in the advanced stages of development. The following stages in ovarian development of adult and of large immature crayfish have been used in this study: (1) oocytes are small (<0.3 mm. in diameter), white in color, and barely visible without magnification; (2) oocytes are somewhat larger (largest oocytes 0.3–0.6 mm. in diameter), white in color, and the anterior lobes of the ovary are turned upwards, due to an increased number of oocytes; (3) oocytes are large (largest oocytes 0.6–1.3 mm. in diameter), white to yellow in color, and the anterior lobes nearly reach the dorsal carapace; and (4) oocytes are very large (largest oocytes > 1.3 mm. in diameter) and yellow to orange in color.

Data of the percentage of females with large, yolky oocytes (stage 4) were obtained during two consecutive years, beginning during the fall of 1961. The number of adult females sampled varied from 3 to 24. The data clearly show an annual cycle for each of the two years sampled (Fig. 1). The lowest frequency of females with large, yolky oocytes in their ovaries occurs during the late summer and early fall months. The frequency rises during late fall and winter months, reaches a peak during early spring months and declines during late spring and summer months as eggs are laid by some females and perhaps as oocytes are resorbed in others.

A new annual period of oocyte formation begins during the summer, because the frequency of large immature animals and adults in stage 2 of ovarian development starts to increase after reaching an annual low in late spring (Fig. 2). Concurrently during the summer, the frequency of adults in the advanced ovarian stages (3, 4) declines to an annual low, but then shows an annual rise as oocytes in stage 2 ovaries mature. The new period of oocyte formation continues during the fall. The progression of oocyte formation and development was observed in five individuals marked and subsequently recaptured one to three months later. One animal, whose ovary failed to show an increase in development of oocytes from November 17 to February 24, was injured and ready to molt on the latter date; perhaps the injuries and molting had inhibited oocyte development. The frequency of females in stage 2 declines to the annual low, as maturation of oocytes continues during winter and spring months. The complete seasonal cycle of oocyte development during one year in Shiloh Cave is known for one marked female. This animal had molted a few days before being marked on August 21, 1961, when its ovary

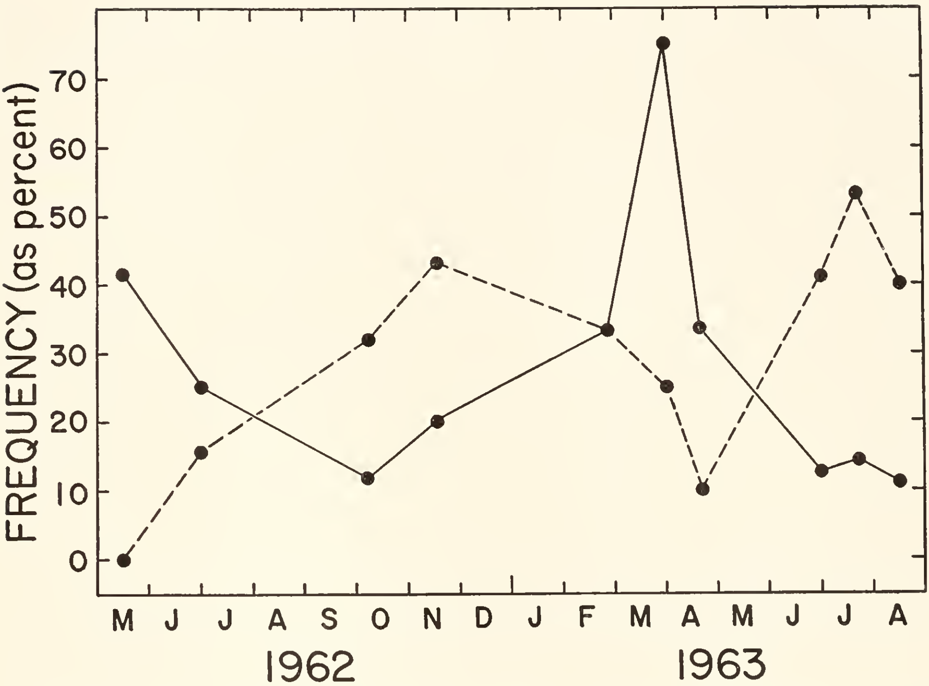

FIGURE 2. The frequency of adult females with the ovary in advanced stages of oocyte development, stages 3 and 4 (solid line), and the frequency of large immature and adult females with the ovary undergoing seasonal proliferation of oocytes, stage 2 (dashed line), in the population of *O. p. inermis* in Shiloh Cave. The number of adult females in samples varied from 4 to 24. The number of adult and large immature females in samples varied from 4 to 32.

was in stage 1. It was in stage 2 on September 23 and was carrying eggs on June 30. The animal was taken to the laboratory (15–17° C.) where it died on August 5. The embryos were nearly ready to hatch and the ovary was in stage 1, completing a one-year reproductive cycle.

Annual changes in the frequency of immature crayfish <18 mm. carapace length present in the population occur as a result of the annual female reproductive cycle (Fig. 1). The frequency of immature animals rises during the summer and early fall as new young leave the adult female. The frequency declines sharply during late fall when new young are no longer added to the population and as larger immature crayfish are becoming adults.

Discussion of Female Reproductive Cycle

Ortmann (1960) claimed that species of crayfishes living in cool streams where the yearly temperature tends to be uniform lose seasonal periods of reproduction. This is not the case for *O. p. inermis* in Shiloh Cave. My data suggest that eggs are laid and carried during spring and summer months, eggs are hatched during summer and fall months, and that most young enter the stream population during late summer and fall. Reports on the occurrence of ovigerous females during other than spring and summer months do not negate this hypothesis. The developmental time of the eggs in *O. p. inermis* is from two to three months. A female laying eggs towards the end of the egg-laying period could still be carrying them during late fall or early winter.

The time of events from the beginning of oogenesis to hatching of the young during the year is similar in *O. p. inermis* as in many epigean species of crayfishes. But there is a difference in the proportion of females laying eggs during any one year. Nearly all the adult females of *O. propinquus* (Van Deventer, 1937) and *O. immunis* (Tack, 1941) lay eggs each season. I suggest that not all adult females of *O. p. inermis* lay eggs each season, because few ovigerous females are found. The probability of finding ovigerous females is high since there is a long developmental time (2–3 months). Also, a small proportion of young relative to the adult population is found. A small percentage of females laying eggs may be an adaptation to the relatively low food supply in Shiloh Cave and may be a means of regulating population size of these crayfish.

Male Reproductive Cycle

Two morphological types of adult males, known as forms I and II, occur in species of the subfamily Cambarinae. These forms are associated with the reproductive cycle and usually alternate during the life of an individual (Hobbs, 1942). Form I males are in the breeding stage while form II adults are not known to mate. An immature male is in form II until the last juvenile molt when it changes to form I and thereafter alternates between form II and form I, when molting, for the remainder of its life. Form I males of *O. p. inermis* in Shiloh Cave have large, curved hooks on the ischiopodite (third most proximal segment) of the second pair of walking legs and small hooks on the ischiopodite of the third pair of walking legs. Form II males in Shiloh Cave have small, blunt hooks on the second pair and no hooks, except on rare occasions, on the third pair of walking legs. There are also differences between the gonopods (copulatory, first abdominal appendages) of the two male forms. In relative terms both tips of each gonopod are longer and pointed in form I males but shorter and blunt in form II males. In addition one of the tips of each gonopod is corneous (hard and dark in color) in form I males; otherwise the entire distal one-fourth of the gonopods in these males remains white, even after the rest of the animal shows post-molt darkening.

Observations on Male Reproductive Cycle

Data of the occurrence of adult males ($\geq$21.5 mm. carapace length) were obtained during four consecutive years in Shiloh Cave (Fig. 3). The number of adult males sampled varied from 7 to 33. The frequency of form I males is lowest during the summer months, increases and reaches a maximum during the fall, and decreases during the late winter and spring months to the summer minimum. The annual wave form is uniform with respect to time of year but does differ in amplitude of the maximum and minimum. The maximum frequencies recorded during the fall of each year from 1960 to 1963 were 85%, 84%, and 97%, while the minimum frequencies for these years were 50%, 50%, and 47%, respectively. Minimum frequencies of 33% and 27% were recorded for the years 1963 and 1964. Data for the years 1960–63 show a rapid increase in form I males with progression of the fall molting period (see results on molting), but the most abrupt change in frequency was recorded during 1964 when the percentage of form I males changed from a low of 27% on August 16 to 67% by August 26. Most of the adult males are in form I by the beginning of November when mating is probably at its peak. After November the trend reverses and males may begin reverting to form II. During 1960–61 there was a decrease in form I males from 80% on December 10 to 50%

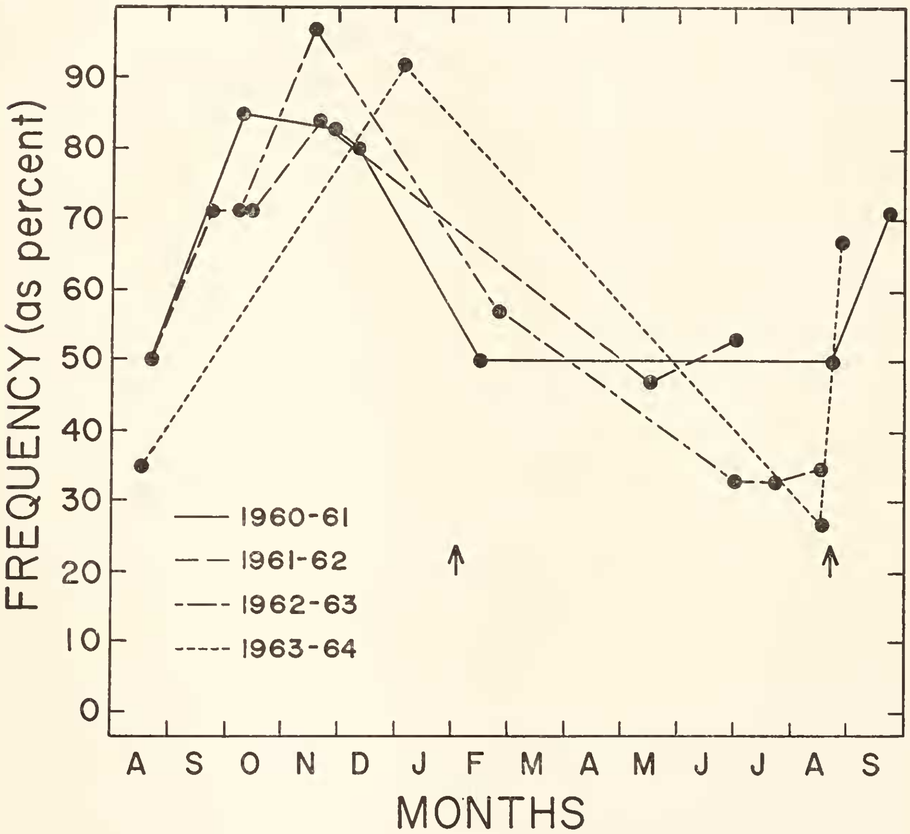

FIGURE 3. The annual change in frequency of form I male, *O. p. inermis* in Shiloh Cave during four successive years. The arrows indicate when the annual frequency decreases in February and increases in August. The number of adult males varied from 7 to 33.

on February 14 and during 1962–63 from 97% on November 17 to 57% on February 24. The frequency of form I males was 92% on January 4, 1964. This is the latest record in the annual cycle I have when adult males were predominantly in breeding form before the annual change in the population to form II. It appears that the initial prominent decrease in form I males occurs at the end of January and beginning of February. On October 7 and November 17, 1962, a total of 18 form I males were marked and only three of these were recovered on February 24.

1964. Probably many of the remaining 15 had already changed to form II. After February the frequency of form I males may continue to decrease to the annual minimum. During 1963 the frequency dropped from 57% on February 24 to 33% by June 30 and continued at this level until the annual fall increase of form I males. It is obvious that, while most adult males are in form I during the fall, not all of them revert to form II during the spring.

The testis of a crayfish is a compound acinar organ, trilobed, and lies immediately ventral to the pericardium. In *C. montanus* acini are proliferated annually, produce a quantity of spermatozoa, and then degenerate (Word and Hobbs, 1958). A few testes were preserved during different months from July, 1962, through August, 1963, to determine whether an annual cycle of spermatogenesis occurs in the cave environment. As the number of testes studied was small (20) the results should be considered preliminary and not entirely conclusive.

Table I

Summary of annual changes occurring in the testes of adult O. p. inermis in Shiloh Cave and correlation of these changes with morphological form

Months	Predominant male form	Condition of testes
June, July, August	II (change from II to I begins during August)	Testes in full seasonal production of spermatozoa; much meiotic activity; many spermatids; great amounts of transforming spermatids and translocation of spermatozoa to collecting ducts.
October and November	I	Spermatogenesis ebbing; large amounts of spermatids, transforming spermatids, translocation of spermatozoa and degeneration of acini.
February	Annual change from I to II begins	Seasonal spermatogenesis essentially over; testes relatively small due to previous massive degeneration of acini; some spermatids; some translocation of spermatozoa.
March, April, May	II	End of seasonal spermatogenesis; few spermatids or spermatozoa remain.

Apparently a new annual season of proliferation of acini and spermatogenesis begins during the summer months (Table I). Perhaps June was the month of 1963 when meiotic activity was principally initiated. A peak of spermatogenesis occurs during July and August. Seasonal spermatogenesis decreases during the fall and is nearly completed by the end of February when many adult males are reverting to form II. There appears to be little spermatogenic activity during the spring months.

Discussion of Male Reproductive Cycle

The population of *O. p. inermis* in Shiloh Cave is similar to many epigean species of crayfishes in having two periods of change in male form during the year. In some species all or most of the adult males in a population change form before and after the mating season: *O. limosus* (Ortmann, 1906), *O. immunis* (Tack, 1941), *O. obscurus* (Ortmann, 1906; Crocker, 1957), *O. propinquus* (Van Deventer, 1937), *C. montanus* (Word and Hobbs, 1958), and *C. longulus* (Smart, 1962). This situation is responsible for the predominance of one form or the other during certain months. However, in *O. p. inermis* not all the males change form twice a year, although the change during the fall is nearly complete. This results in the occurrence of form I males throughout the year—over 90% during late fall and winter and about 30–50% during spring and summer. Aiken (1965) reported a similar result for *O. virilis.* Some authors have interpreted data showing both male forms of a species occurring during most of the year to mean that no annual periods of reproduction occur in that species. Such an interpretation does not apply to *O. p. inermis* in Shiloh Cave.

The time of events in the annual spermatogenic cycle during the year appears to be similar, in some respects, to that described by Word and Hobbs (1958) for the epigean species, *C. montanus.*

The Annual Molting Cycle

Previous knowledge of molting cycles in cave crayfish is meager. Hay (1903) inferred from limited observations that an ecdysis of *O. p. pellucidus* occurs in Mammoth Cave, Kentucky, about the same season as in epigean crayfish. Banta (1907) believed that *O. p. inermis* from Indiana molted from two to four or five times a year, depending on size. Putnam (1877) observed a specimen from Mammoth Cave molt twice during six months in the laboratory, although several appendages had been injured previously. Molting in cave crayfish is more difficult to assess than reproductive activities because in addition to effects of light, darkness and temperature on molting in crustaceans, starvation may inhibit molting (Roberts, 1957), multiple leg autotomy sometimes promotes molting (Passano and Jyssum, 1963) and molting is inhibited in ovigerous females (Tack, 1941). For the present analysis of molting in cave crayfish the discussion will be limited to adult and large immature animals.

Observations on Annual Molting Cycle

The best criterion of molting in male crayfishes of the subfamily Cambarinae is the change in ratio of the form of the adults. There are two such changes each year in the Shiloh Cave population (Fig. 3). One occurs principally during February and March (the spring molting period) and the other principally during August and September (the fall molting period). Relative hardness and darkness of the exoskeleton also proved to be valuable criteria in studying the molting cycle of cave crayfish. Pooled data from these sources show the same two annual periods of molting for both sexes (Fig. 4) that were discovered for males from the changes in ratio of male form. These two periods are the predominant times of molting, but some adult and large immature animals are found molting during other months of the year (Fig. 4). The spring molting period is more pronounced in males than females because many of the adult females probably bypass this period and do not molt. The fall molting period is well pronounced in females, and most of them probably molt at this time.

Although both periods are well demonstrated in males, it should be emphasized that these are phenomena of the population as a whole and do not indicate that every male molts twice every year. Small and medium-size animals perhaps do,

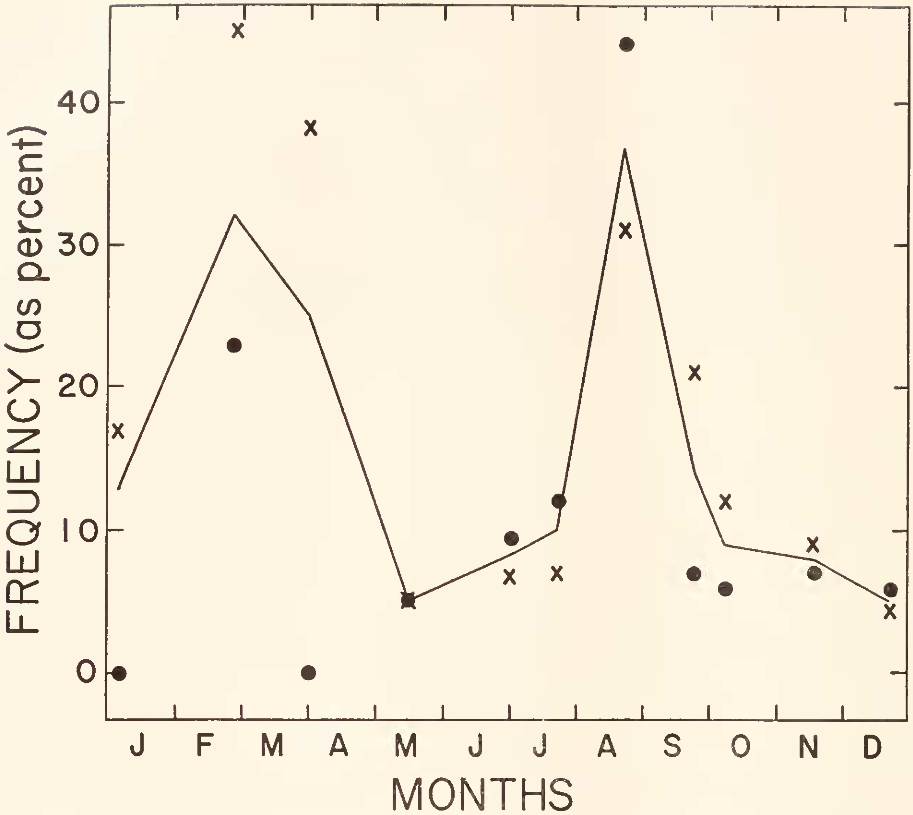

FIGURE 4. Annual molting periods of large immature and adult crayfish, *O. p. inermis*, in the Shiloh Cave population. Data obtained during the years 1962, 1963 and 1964 have been used to construct this graph. The number sampled varied from 12 to 76. The points for each date represent pooled data from the various indicators of molting (ecdysis, relative hardness and relative darkness of the exoskeleton). Females (●), males (×), males and females combined (——).

but it is clear that the larger animals do not. Some large form II males were found during the winter intermolt period, indicating that they had bypassed the fall molting period; but some large form I males with hard, dark exoskeletons occurred during the summer intermolt period, and these animals probably had not molted since the previous fall.

Discussion of Annual Molting Cycle

In crayfishes living in the central and northern United States the two molting periods are usually less than four months apart, thereby creating short summer and long winter intermolt periods. During one year of Van Deventer's study of *O. propinquus* both molting periods occurred within one month's time. In Shiloh Cave crayfish the two annual peaks of molting of adult and large immature animals are six months apart, with intermolt periods of three months. All of the present data indicate that the peaks of molting occurred at the same times of the year during this study but with differences occurring in the frequency of molting on the same date during different years.

General Discussion

It is clear that the cave-dwelling crayfish *O. p. inermis* has annual cycles of reproduction and molting. The fact that some molting does occur throughout the year suggests that these cycles, at least the molting cycle, are not completely controlled by a seasonal factor in the environment. But something does induce the majority of the animals to molt during two periods of the year and induces the majority of adult males to be in breeding form during the fall. Perhaps a basic endogenous rhythm is present but some seasonal factor in the environment is acting to synchronize molting and reproduction, although imperfectly, in the population. Is there a factor, in the absence of photoperiod and large temperature changes in the environment, which is seasonally variable enough to account for these consistent annual periods?

Gradual changes in organic material or water level, or the occurrence of flooding (rapid changes of water level, increased current and increased turbidity) perhaps could act to synchronize or modify biological cycles in Shiloh Cave, but no quantitative data on the first two variables were obtained. According to Ginet (1960) dissolved organic material is greatest in French caves during late summer and early fall and lowest during the winter. Water levels in seven caves of the central United States are highest during late winter and early spring (Poulson, 1964). However, these may be subtle factors depending on the particular cave system. The occurrence of flooding in caves is well known; in Shiloh it has been observed on December 18, 1961, March 31, 1962, and March 31, 1963—the latter date was near the end of an unusually heavy flood, with high water level continuing through April. Flooding is certainly an adverse environmental factor for rearing of young crayfish, and selection might favor egg-laying and hatching at times other than the possible late November to April flooding season in Southern Indiana caves. The most favorable period for egg-laying in cave crayfish would be towards the end of the flooding season as this would allow time for development and growth of the young to a point where they could cope successfully with the rushing water of the next flooding season. This appears to be the situation in Shiloh Cave and apparently in several other caves where some data are available.

A season of particularly strong flooding perhaps would induce many females either to resorb their oocytes or to lay eggs in a relatively short period of time. This may have been the case during the spring of 1963 when the frequency of females with large, yolky oocytes dropped sharply, reaching 0 by July 21 (Fig. 1). On the other hand, after a season of weak flooding one might expect to find less of an inducing effect on the females and a subsequent lengthening of the period when females either resorb oocytes or lay eggs. During the 1961–62 reproductive cycle weak floods were noted on December 31 and March 31. The decrease in frequency of females with large, yolky oocytes was much slower than in 1963 and was prolonged through the summer and into the fall, finally reaching 0 during September (Fig. 1). The egg-laying period was thereby shortened during 1963 in reference to the period during 1962.

An environmental factor that regulates the annual cycle of gametogenesis and molting in male cave crayfish is unknown. In the Shiloh Cave population each molting cycle is approximately six months in duration; each molt period and each intermolt period have durations of about three months. The spring molting period occurs during the time when flooding may occur, which superficially would appear to give the animals considerable disadvantage in the struggle to survive a severe flood. Observations on March 31, 1963, show clearly that they can molt and survive during a flood. There is no obvious change in the environment to account for the increase in molting and change in male form during August of each year. A possible explanation of such a phenomenon is that the annual cycles of these males are synchronized by the environment some time before August, perhaps by the spring floods.

Annual reproductive periods have been reported for other cavernicolous animals. The cave fish, *Amblyopsis spelaea,* living in the same limestone area as *O. p. inermis,* lays eggs during high water from February through April; the young leave the maternal gill cavity and appear in the streams during late summer and early fall (Poulson, 1963). *A. spelaea,* then, is not dissimilar in its season of production of young from *O. p. inermis.* In France the amphipod *Niphargus orcinus virei* carries eggs throughout the year, but there is a maximum period during the late spring and summer (Ginet, 1960). Ginet concludes that an endogenous rhythm of reproductive activity not directly bound to fluctuations in the environment may be present. This suggests to me that the cycles of reproductive activity of individual amphipods are imperfectly regulated by the environment, with the consequent poor synchronization of reproduction in the population and much overlapping of the individual cycles. A lack of seasonal reproduction has been reported for several cavernicolous animals. Deleurance and Deleurance (1964) found that the proportion of ovigerous female beetles of the subfamilies Bathysciinae and Trechinae shows annual variations, but the variations are not seasonal. Mitchell (1965) found recently pupated beetles, *Rhadine subterranea,* at all times of the year, indicating that seasonal reproduction is absent. Both studies do not show reproductive activities for individuals. Individuals might well have an annual rhythm of reproduction but if the environment does not induce individuals to reproduce seasonally, reproduction will probably occur in a population throughout the year because of individual variation in length of the annual cycles. Perhaps flooding, which may have an effect on seasonal reproduction of aquatic cave animals, has no effect on reproduction of the terrestrial animals.

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Summary

1. Annual periods of reproduction and molting of *Orconectes pellucidus inermis* in Shiloh Cave, Indiana, have been observed. Both sexes of *O. p. inermis* in Shiloh

Cave appear to initiate a new annual period of gametogenesis in early summer. The number of adult females whose ovary contains large, yolky oocytes is declining to an annual minimum and the majority of adult males are in form II. Eggs are being laid and hatched and young crayfish are leaving the adult and entering the stream population. Oocyte formation and growth, spermatogenesis, and maturation of spermatozoa continue during the fall months. Many adult and some large juvenile males molt to form I. During fall months most adult males are form I, they have large supplies of spermatozoa, and mating presumably occurs. Oocytes of some females begin to mature and there is an increase in the proportion of females having the ovary filled with large, yolky oocytes.

During late winter and early spring months spermatogenesis and oogenesis decline to an annual minimum, that is reached during the spring months. Concurrently many adult males molt to form II. Not all the adult females molt at this time of year. The proportion of females with large, yolky oocytes in their ovaries increases to the maximum during the spring.

2. Evidence for environmental synchronization of the reproductive and molting cycles of individuals in the population is discussed.

3. Occurrence and absence of seasonal periods of reproduction reported by other investigators in a few species of cavernicolous animals are discussed.

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